A New Mutation (*t-int*) Interacts With the Mutations of the Mouse *T/t* Complex That Affect the Tail

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ABSTRACT

A new recessive mutation affecting tail length is described. It is unlinked to the *T/t*-complex on chromosome *17* and yet it shows cumulative phenotypic effects with several *t*-complex mutations: *T*, T^{Cu}, *tct* and Fu^{hi}. It does not interact with five different nonchromosome *17* mutations that affect tail length. Thus, *t-int* is a tail modifier with surprisingly specific interactions.

THE proximal part of the *t*-complex on chromosome *17* in the mouse contains two genes known to affect tail length (ARTZT, BENNETT and SHIN 1985). One is the semidominant *Brachyury* mutation (*T*) which causes a short tail in heterozygotes and is lethal at midgestation in homozygotes. The other mutation *tct* (the "tail interaction" factor formerly called *t-int* and t^7) is present only in *t*-haplotypes and, curiously, by itself has no phenotype in the heterozygous or homozygous condition. It is recognized by virtue of its interaction with *T* to produce tailless mice in compound heterozygotes (*T/tct*). In a formal sense, these two genes behave as alleles since they segregate from one another without recombination; however, since all naturally occurring *tct* factors exist within an inversion (HERRMANN *et al.* 1986) it is not known whether *T* and *tct* are unilocal.

Both the short tailed and the tailless phenotypes are very sensitive to modifiers of tail length in the genetic background. For example, on the BALB/c background genetically short-tailed mice (*T/+*) tend to have very short tails, no more than one-third normal length, are frequently completely tailless and occasionally show spina bifida, while genetically tailless mice (*T/tct*) often lack some sacral vertebrae, and may have severe spina bifida aperta. On the C3H background, on the other hand, *T/+* mice have tails that are often 9/10 of normal length, and frequently overlap with normal. The number of the background modifiers in these strains is not known, nor have any been specifically identified. We report here the serendipitous discovery of a very specific modifier gene which does not appear to be linked to chromosome *17*. We have called the new gene *t-int* since it appears to be unlinked to the *t*-complex. This symbol was previously used for the mutation now designated *tct*, but was discarded when the nomenclature committee decided that all *t*-linked tail interactive genes are to be called *tct*. Alone in heterozygotes or homozygotes *t-int* has no phenotype but has surprisingly discrete interactions with both *tct* and *T*, and also Fu^{hi}, another tail mutation located in the *T/t* complex. It does not interact with several other dominant and recessive tail mutations located on other chromosomes.

METHODS AND RESULTS

A single short-tailed (length 50% of normal) mouse was observed in a stock maintained homozygous for a viable proximal partial *t*-haplotype (t^{w111}) and the linked marker *tufted* (*tf*) (*tct tf/tct tf*). When this male was mated to wild-type females, only normal tailed progeny resulted (Table 1, line 1). He was subsequently crossed to some of his normal tailed tufted sibs and produced both normal and short-tailed progeny like himself. When these short-tailed animals were bred together they produced normal-tailed, short-tailed and completely tailless progeny in proportions that resembled a 1:2:1 ratio (Table 1, line 2). The tailless mice, when mated together, bred true giving only tailless offspring (Table 1, line 3). Thus the mutation appeared to be recessive but interacted in an additive way with homozygous *tct* to give short and tailless mice. Our simplest working hypothesis, as shown in Table 2, was that in *tct* homozygotes one dose of the new mutation results in a short-tailed phenotype, while two doses produce taillessness. To further clarify the situation, an F_2 linkage test was performed between *t-int* and *tf*. Tailless homozygous mice (*tct tf/tct tf, t-int/t-int*) were crossed to C3H (+/+, +/+) and the resulting normal tailed F_1 mice (++/*tct tf*, +/*t-int*) were mated together. The tails of the progeny were scored at birth and a proportion of them were raised to score for *tf* at 28 days (Table 1, lines 4 and 4a). Clearly, taillessness is absolutely asso-

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TABLE 1

Interaction of *t-int* with *T* and *tct*

	Parental genotypes		Normal tails	Short tails	Tailless
1.	*tct tf/tct tf, +/t-int* short tail, tufted	× +/+, +/+ normal tail, nontufted	77	0	0
2.	*tct tf/tct tf, +/t-int* short tail, tufted	× *tct tf/tct tf, +/t-int* short tail, tufted	22	32[a]	18
3.	*tct tf/tct tf, t-int/t-int* tailless, tufted	× *tct tf/tct tf, t-int/t-int* tailless, tufted	0	0	175
4.[b]	*+/tct tf, +/t-int* normal tail, nontufted	× *+/tct tf, +/t-int* normal tail, nontufted	168	93[a]	23
4A.	normal tail nontufted 55	normal tail tufted 10 / short tail nontufted 27	short tail tufted 14	tailless nontufted 0	tailless tufted 17
5.	+/+, *t-int/t-int* normal tail, nontufted	× *tct tf/tct tf, t-int/t-int* tailless, tufted	0	55[a]	0
6.[b]	*+/tct tf, t-int/t-int* short tail, nontufted	× *+/tct tf, t-int/t-int* short tail, nontufted	44	143[a]	40
7.	+/+, *t-int/t-int* normal tail, nontufted	× +/+, *t-int/t-int* normal tail, nontufted	61	0	0
8.	*T*/+, +/+ short tail, nontufted	× +/+, *t-int/t-int* normal tail, nontufted	60	0	61[c]
9.	*T*/+, *+/t-int* tailless, nontufted	× +/+, *t-int/t-int* normal tail, nontufted	11	0	18[c]
10.	T^{Cu}/+, +/+ tailless, nontufted	× +/+, *t-int/t-int* normal tail, nontufted	38	0	35[d]

[a] Tail length was approximately 50% of normal, and was therefore clearly distinguishable from normal and tailless phenotypes.
[b] Departure from the expected ratio is not yet explained.
[c] Variable tailless phenotype ranging from complete taillessness to stumps 2–4 mm measured at birth.
[d] All have sacrococcygeal spina bifida of varying severity.

TABLE 2

Expected proportions and phenotypes of the F_2 progeny

Proportion of total offspring	Genotype at *tct tf*	Genotype at *t-int*	Phenotype
1. 1/16	++/++	+/+	Normal tail, nontufted
2. 2/16	++/++	*+/t-int*	Normal tail, nontufted
3. 1/16	++/++	*t-int/t-int*	Normal tail, nontufted
4. 2/16	*++/tct tf*	+/+	Normal tail, nontufted
5. 4/16	*++/tct tf*	*+/t-int*	Normal tail, nontufted
6. 2/16	*++/tct tf*	*t-int/t-int*	Short tail, nontufted
7. 1/16	*tct tf/tct tf*	+/+	Normal tail, tufted
8. 2/16	*tct tf/tct tf*	*+/t-int*	Short tail, tufted
9. 1/16	*tct tf/tct tf*	*t-int/t-int*	Tailless, tufted

ciated with *tf* (17 of 17), whereas shortness is not (14 of 41 short-tailed mice were also tufted).

The simplest interpretation is that we have identified an unlinked gene that when homozygous produces taillessness in mice that are also homozygous for *tct*. Furthermore, the "short" phenotype apparently occurs when any three of the four genes at the two loci are mutant, *i.e. tct/tct, +/t-int*, or *+/tct, t-int/t-int*. Thus, barring recombination between *tct* and *tf* (8–10 cM), half the shorts will be tufted and half will not (see Table 2 for all genotypes). The proportion of tail phenotypes at birth (Table 1, line 4) does not absolutely fit this hypothesis: in a sample of 284, the numbers of normal, short and tailless expected are 195:71:18, and the observed phenotypes were 168:93:23 ($\chi^2 = 11.9$, $P = 0.0027$). However, this statistical deviation may result from complicating factors too subtle to deal with easily; possible causes we have considered include another modifier gene segregating in the genetic background, small inter-male variations in transmission ratio of t^{w111}, or altered transmission of *t-int* itself. Models can be constructed to take into account any one or a combination of these factors, but they are too cumbersome to present here in detail.

In order to evaluate our original interpretation it was necessary to demonstrate that the homozygotes for *t-int* alone (+/+, *t-int/t-int*) had a normal tail phenotype. To do this normal-tailed F_2 mice that were non-tufted and therefore either ++/++ or *++/tct tf* with respect to the relevant markers on chromosome *17*, and of three possible genotypes (+/+, *+/t-int* or *t-int/t-int*) at the other locus (Table 1, line 4A) were test crossed to the tailless tufted doubly homozygous stock *tct/tct, t-int/t-int*. Of the possible F_2 genotypes only those that were wild type at the *tct* locus but homozygous for *t-int* (+/+, *t-int/t-int*) could produce progeny consisting solely of short-tailed mice, because their offspring would be obligate heterozygotes at the *tct* locus and obligate homozygotes at the *t-int* locus.

Thus all their progeny would carry three copies of mutant genes and be short-tailed; no normal-tailed progeny were expected because that phenotype requires two copies of +, and likewise no tailless phenotype was expected because that requires all four mutant genes. One female produced no normal-tailed, 55 short-tailed and no tailless progeny, and was thus defined as +/+, *t-int/t-int* (Table 1, line 5). Her short-tailed offspring from this test were mated together (Table 1, line 6) and produced nontufted normal-tailed offspring who, like their grandmother, were genotypically only +/+, *t-int/t-int*. A line of these has been established which breeds true for the normal-tailed and non-tufted phenotype (Table 1, line 7). Thus *t-int* has no phenotype in the homozygous condition in the absence of *tct*.

Since it appeared that *t-int* has a subtractive effect on tail length, its interaction with dominant *T* mutations was tested. Normal-tailed *t-int/t-int* homozygous males were crossed to mice carrying our standard *Brachyury* chromosome maintained on a background (BTBRTF/Nev) selected for more than 30 years for consistent 50% tail length in *T*/+ heterozygotes. Thus all progeny from this cross (*T*/+, +/+ × +/+, *t-int/t-int*) are also *t-int* heterozygotes. Sixty normal-tailed and 61 virtually tailless mice were obtained (Table 1, line 8), demonstrating that *t-int* interacts with *T* in a manner similar to *tct*. There was no phenotypic overlap in tail length with mice from control litters in which *T* alone was segregating. Backcrosses of these tailless mice to *t-int* homozygotes did not, however, produce a more severe phenotype (Table 1, line 9).

Another dominant *T* mutation designated T^{Cu} (*Curtailed*) uniformly produces a tailless (instead of short-tailed) phenotype in T^{Cu}/+ heterozygotes. T^{Cu} also has the property of producing sacro-caudal spina bifida in compound T^{Cu}/tct heterozygotes (SEARLE 1966; D. BENNETT, unpublished data). T^{Cu}/+ when crossed to +/+, *t-int/t-int* produced 38 normal-tailed and 35 tailless mice which all had spina bifida (Table 1, line 10). Thus, *t-int* interacts not only with *tct* but also with *T* and T^{Cu} to increase the severity of their phenotypes.

Since *t-int* interacts both with dominant *T* (*T* and T^{Cu}) and recessive *tct* mutations, we tested whether the interaction is specific to T/t complex genes or whether *t-int* generally modifies mutations affecting the tail. The first candidate examined was another dominant tail mutation, "*Kinky*" (Fu^{ki}) located on chromosome *17*. This is an allele of "*Knobbly*" (Fu^{kb}) (JACOBS-COHEN *et al.* 1984) which is located in the t-complex between *T* and *tf* (LYON 1978). It is, however, not allelic to *T* and its phenotype is not altered by *tct* (JACOBS-COHEN *et al.* 1984). DUNN and CASPARI (1945) reported no appreciable phenotypic interaction of *T* and Fu^{ki} although on more recent genetic backgrounds, cumulative phenotypic effects have been seen (D. BENNETT, unpublished results). Thus, if the interaction of *t-int* with *tct* and *T* is absolutely specific, one might expect that *t-int* would not interact with Fu^{ki}. We tested the Fu^{ki} from our colony. None of the 30 F_1 progeny (Fu^{ki}/+, +/*t-int*) had other than the standard parental phenotype. However, we backcrossed the F_1 to +/+, *t-int/t-int* with the expectation that one half of the Fu^{ki} progeny would be of the genotype *t-int/t-int* and might have an altered phenotype. The results were 14 normal, 3 kinky, and 3 short tails! The phenotypes in the latter two categories were clearly distinct: the kinky tails were kinky but long (10–12 mm at birth), while the short tails measured 4–5 mm. Thus homozygosity at *t-int* has a distinct negative impact on the heterozygous phenotype of Fu^{ki}.

Several tail mutations not on chromosome *17* were obtained from the Jackson Laboratory, Bar Harbor, Maine. Three dominant mutations were tested: *Loop tail* (*Lp*) on chromosome *1*, *Tail short* (*Ts*) on chromosome *11*, and *Pin tail* (*Pt*) on chromosome *4* (PETERS 1986). Neither F_1 mice (*Dominant*/+, +/*t-int*) nor the progeny of backcrosses of F_1 mice to *t-int/t-int* (where one half of the affected classes should have been *Dominant*/+, *t-int/t-int*) showed any discernible change from the phenotype typical of the original mutants (Table 3).

Two recessive tail mutations were tested, "*tail kinks*" (*tk*) on chromosome *9* and "*pudgy*" (*pu*) on chromosome *7*. For *tk* we crossed *tk/tk* females to *t-int/t-int* homozygotes to obtain mice of the genotype +/*tk*, +/*t-int*. These would be expected to have normal tails since both genes are recessive, but one quarter of the progeny from backcrosses to *t-int/t-int* (+/*tk*, +/*t-int* × +/+, *t-int/t-int*) might be expected to have an abnormal tail phenotype if the two genes interact. Forty-one backcross progeny were examined and all had essentially normal tails except for an occasional blunt tip.

In the case of *pu*, since homozygous females are poor breeders, we started with a heterozygote. Thus the F_1 mice were unknown for their genotype at *pu* (+/*pu*?, +/*t-int*). Four such females were backcrossed to +/+, *t-int/t-int* males. Their respective progeny sample sizes were: 22, 23, 30 and 34 for a total of 109 essentially normal-tailed backcross mice. One half of the four females are expected to carry *pu* and half of their offspring could be expected to be homozygous *t-int/t-int*. Thus statistically, of the 109 progeny, one quarter could be expected to have an abnormal tail phenotype if *pu* interacted with *t-int*. It is of course possible that none of the four F_1 females carried *pu*. Among the 109 there were 12 occasional blunt tails (87% normal length measured at birth) evenly distributed among the progeny of the four females. Since this number (11%) did not fit any genetic ratio, it was

TABLE 3

Interaction of *t-int* with other mutations

Chromosome	Gene	Parental genotypes	Progeny		
			Normal tails	Anticipated tail mutations[a]	Unexpected phenotypes
17	Fu^{ki}	Fu^{ki}/+, +/+ × +/+, *t-int/t-int*	27	30	0
		Fu^{ki}/+, +/*t-int* × +/+, *t-int/t-int*	14	3	3
1	*Lp*	+/+, *t-int/t-int* × *Lp*/+, +/+	5	7	0
		Lp/+, +/*t-int* × +/+, *t-int/t-int*	15	13	0
11	*Ts*	+/+, *t-int/t-int* × *Ts*/+, +/+	17	11	0
		Ts/+, +/*t-int* × +/+, *t-int/t-int*	35	36	0
4	*Pt*	*Pt*/+, +/+ × +/+, *t-int/t-int*	7	2[b]	0
		+/+, *t-int/t-int* × *Pt*/+, +/*t-int*	42	24[b]	0
9	*tk*	*tk/tk*, +/+ × +/+, *t-int/t-int*	11	0	0
		+/*tk*, +/*t-int* × +/+, *t-int/t-int*	41[c]	0	0
7	*pu*	+/*pu*, +/+ × +/+, *t-int/t-int*	32	0	0
		+/*pu*?, +/*t-int* × +/+, *t-int/t-int*	109[d]	0	0

[a] Based on no interaction between *t-int* and the mutation under test.
[b] The deficiency of *Pt* heterozygotes is presumed to be due to variable penetrance (HOLLANDER and STRONG, 1951).
[c] Seven of the normal tails were blunt (87% normal length).
[d] Twelve of the normal tails were blunt (87% normal length).

assumed that this phenotype resulted from the combination of *t-int/t-int* and the genetic background of *pu*. Thus, for the two recessive mutations examined +/*r*, *t-int/t-int* has essentially a normal-tailed phenotype.

DISCUSSION

We have defined a modifier of the *T* and *tct* tail mutations with surprisingly discrete phenotypic effects. Three mutant copies of either *tct* on chromosome *17* or the unlinked *t-int* produce a short-tailed phenotype while homozygotes for either mutation alone have no discernible abnormality of tail phenotype. Four mutant copies at the two loci produce an absolutely tailless phenotype, thus their effects appear to be additive. In addition, *t-int* intensifies the phenotype of both *T* and T^{Cu}, in the former case from a short tail to virtual taillessness and in the latter from taillessness to spina bifida.

The genetic interaction of *t-int* with *T* and *tct* was surprisingly specific for tail mutations within the *t*-complex. When homozygous the *t-int* mutation also interacts with another more distal chromosome *17* tail mutation (Fu^{ki}) to produce a more extreme phenotype. However, three other unlinked dominant tail mutations and two unlinked recessive ones appear to have no interaction with *t-int*.

This is the first instance of a mutation unlinked to chromosome *17* that interacts with mutations at the *t*-complex. However, there are two other examples of mutations arising in *tct/tct* mice that produce short tails. Vojtiskova *et al.* (1976) reported a new *t*-haplotype, t^{AE5}, that derived from a viable proximal partial t-haplotype t^{11}. Homozygotes for t^{AE5} were short-tailed, while +/t^{AE5} were normal-tailed and *T*/t^{AE5}, as expected, were tailless. On the hypothesis that t^{AE5} might have carried an unlinked mutation, we tested it for linkage to *tf* and it was indeed linked at about 11 cM from *tf* (K. ARTZT, unpublished data).

A second case arose in our colony in another partial haplotype, t^{46}. We could construct homozygotes that bred true for short tails but the mutation proved to be a noninteractive and unlinked recessive mutation and was discarded in 1980 (K. ARTZT, unpublished data).

Thus, *t-int* is the first mutation to be described that specifically interacts with three different mutations in the *t*-complex. One could speculate that *t-int* could be considered a misplaced copy of *tct* since the additive effects of the two mutations imply they operate on the same developmental gradient. This question can most readily be approached when molecular probes to *tct* or regions closely flanking it become available.

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